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Analysis of a diallel cross to estimate effects of crossing on resistance to enteric septicemia in channel catfish, *Ictalurus punctatus*

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Abstract

The trend in commercial channel catfish, *Ictalurus punctatus*, production is toward increased stocking densities and feeding rates, and is often accompanied by disease problems. Enteric septicemia, caused by the bacterium, *Edwardsiella ictaluri*, is responsible for many disease losses in channel catfish cultured in the southern United States. A complete diallel cross among three strains, Red River, Norris, and M × K (Marion × Kansas), was used to estimate effects of crossing on resistance to *E. ictaluri*. Juveniles (mean weight 15.2 ± 3.8 grams) from all nine crosses were challenged by immersion, and survival determined 28 days following bacterial exposure. Mean survival of all crosses was 62.2% and ranged from 35.8% in the M × K female × Norris male cross to 90.0% in the Norris female × M × K male cross. Estimates for heterosis, line, maternal, reciprocal effects, and general combining ability corrected for maternal effects were obtained from contrasts among appropriate mean squares. Average, line or specific heterosis effects were not significant suggesting that crossbreeding these strains would not increase disease resistance due to heterosis. Significant line effects in Norris and M × K strains demonstrated differences for disease resistance in these strains. Significant maternal effects conveyed increased disease resistance to offspring in the Norris strain. Significant general combining ability indicated additive genetic differences for enteric septicemia resistance in Norris and M × K strains.

Keywords: *Ictalurus punctatus*; Enteric septicemia

1. Introduction

Channel catfish, *Ictalurus punctatus*, farming began over 30 years ago and has become one of the most successful aquaculture enterprises in the United States. The most recent

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survey showed 64 000 ha of ponds in production with 202 million kg of catfish processed in 1993. Approximately 90% of the commercial production is located in the southeastern USA in Mississippi (60.0%), Arkansas (12.6%), Alabama (11.4%), and Louisiana (6.3%) (USDA, 1992).

Disease losses in channel catfish culture currently account for between 20 and 95% of total losses (USDA, 1990). Enteric septicemia of catfish (ESC), caused by the bacterium *Edwardsiella ictaluri* (Hawke et al., 1981), is a highly virulent, systemic disease responsible for about 50% of all channel catfish disease losses in the southeastern United States. ESC is presently the most serious disease in the United States catfish industry, and occurs primarily across the southeastern United States. As the catfish industry continues to expand in the United States, ESC will continue to be a significant problem and will likely develop in geographical regions where channel catfish have been imported (Plumb, 1993).

Development of disease resistant stocks of channel catfish through selective breeding has the potential to provide important contributions to commercial catfish farming. Early evaluations of catfish stocks for disease resistance are limited to a few research studies demonstrating phenotypic variation among strains for resistance to viral, bacterial, and parasitic pathogens (Dunham and Smitherman, 1987). Recent studies have shown significant phenotypic variation in *E. ictaluri* resistance among channel catfish strains and families indicating the potential for genetic variability and improvement through selection (Wolters and Johnson, 1994), however, one generation of selection for resistance to bacterium did not improve resistance in Kansas strain channel catfish (Dunham et al., 1994). Crossbreeding has been used to improve growth rate and increase disease resistance (Dunham and Smitherman, 1987). No studies have been conducted on the effects of crossing on resistance to ESC in channel catfish. It may be possible to identify channel catfish strains and crossbreds with increased ESC resistance and estimate genetic effects from crossbreeding.

2. Materials and Methods

2.1. Experimental fish

Juvenile channel catfish (mean weight = 15.2 ± 3.8 grams; age 6 months) from three strains were used in a complete diallel cross model (Gardner and Eberhart, 1966) to evaluate effects of crossing on susceptibility to infection with *E. ictaluri*. Channel catfish strains were designated as: Red River, originally collected in 1988 from the Red River, North Dakota (Hudson Bay drainage); Norris, purchased from Norris Fish Farm, Cash, Arkansas with parentage described by Dunham and Smitherman (1984), and M × K, crossbred Marion × Kansas F_1 broodfish (Dunham and Smitherman, 1984). Strains were maintained in ponds at the USDA Catfish Genetics Research Unit (CGRU), Stoneville, Mississippi.

Three-year old broodfish from each strain were paired in 80 l spawning tanks and induced to ovulate with daily injections of carp pituitary extract (4.4 mg kg^{-1}). When fish began ovulating, eggs were handstripped and fertilized with sperm from macerated testes of donor males (Dupree et al., 1969; Wolters et al., 1981) to make the appropriate nine crosses. Fish from three different full-sib families within each cross of the 3 × 3 diallel were pooled and grown at the CGRU in indoor 160 l tanks supplied with wellwater (26°C, pH 8.6, > 5.0

mg l^{-1} dissolved oxygen, <17 mg l^{-1} total hardness, 383.8 mg l^{-1} Cl^-, 410 mg l^{-1} alkalinity, 1.5 mg l^{-1} total ammonia–nitrogen, and 0 mg l^{-1} nitrite–nitrogen) at a flow rate of 8 l min^{-1}. Fish were fed a sinking trout ration until fish reached approximately 7 cm and then switched to a 41% floating catfish fingerling ration. Fingerlings had no prior history of exposure to *E. ictaluri* and pre-challenge serum antibody levels were negative using an indirect enzyme linked immunosorbent assay (ELISA) technique (Waterstrat et al., 1989).

2.2. *Edwardsiella ictaluri challenge*

An *E. ictaluri* isolate from a natural disease outbreak of ESC was obtained from the Fish Disease Diagnostic Laboratory, Stoneville, Mississippi. All isolates were identified by biochemical characteristics described by Hawke (1979). To assure virulence prior to the challenge tests, the isolate was propagated on blood agar at 25°C for 24 h, inoculated into brain heart infusion (BHI) broth at 25°C for 24 h, pelleted, and resuspended in Hanks' balanced salt solution. Aliquots (0.1 ml) were injected intraperitoneally into ten channel catfish (15–20 cm) reared in a 120 l aquarium maintained at 25°C with wellwater at 1 l min^{-1}. *Edwardsiella ictaluri* isolates from the brain and posterior kidney of ten dead or dying fish were used to inoculate two tubes containing 10 ml BHI broth and incubated for 24 h at 25°C. These two tubes were used to inoculate two 2 l rotating flasks containing 1 l of BHI broth incubated for 24 h at 25°C. Bacterial concentrations at 24 h were determined by replicate plate counts.

A total of 300 fish from each cross were stocked into six replicate 120 l aquaria with 50 fish per aquaria. Flow rates and water temperatures were maintained at 1 l min^{-1} and 25°C. Fish were stocked into test aquaria and acclimated for 7 days prior to the challenge, and consuming feed at 3% of the body weight per day at the time of challenge. Fish were exposed to *E. ictaluri* by lowering the water level in all 54 aquaria to 24 l and adding 10 ml of culture to yield approximately 1.2×10^6 bacteria ml^{-1}. After a 10 min, static exposure period, water flow was resumed and aquaria were allowed to refill to the original volume.

Morbidity, mortality, and cause of death was monitored and recorded daily. Five dead fish from each set of six aquaria per cross were necropsied daily. Fish were tested for *E. ictaluri* by the indirect fluorescent antibody technique (IFA) technique (Ainsworth et al., 1986). Bacterial isolates were identified from biochemical characteristics to confirm the cause of death from *E. ictaluri* (Hawke, 1979). Challenge tests were terminated 28 days after disease exposure.

An analysis of variance was performed to determine if cross as a main effect caused significant variation on survival (GLM procedure: SAS Institute Inc., 1988). Mean mortality of replicate aquaria from each cross were compared with a series of estimate statements in the GLM procedure to estimate average heterosis, line heterosis, specific heterosis, maternal effects, line effects, reciprocal effects and general combining ability and declared significant at $\alpha < 0.05$ (Eisen et al., 1983).

3. Results

Death of channel catfish in test aquaria began approximately 7 days after exposure to *E. ictaluri* bacteria and continued for approximately 10–14 days. All necropsied fish were IFA positive for *E. ictaluri* demonstrating the presence of systemic bacteria.

Table 1
Mean ± SE survival (%) of channel catfish, *Ictalurus punctatus*, from a diallel cross of Red River, Norris, and M × K strains following immersion challenge with *Edwardsiella ictaluri*

	Female Parent		
	Red River	Norris	MxK
Male parent			
Red River	56.5 ± 3.9	68.4 ± 5.8	68.7 ± 4.0
Norris	57.9 ± 7.8	52.5 ± 7.3	35.8 ± 5.8
M × K	60.8 + 4.6	90.0 + 1.5	68.9 + 2.6

Table 2
Estimates(± SE) of genetic effects for survival of channel catfish, *Ictalurus punctatus*, from a diallel cross among Red River, Norris, and M × K strains

Parameter	Estimate ± SE
Line effects	
Red River	3.4
Norris	−28.4*
M × K	25.0*
	(± 5.5)
Average heterosis	4.3
	(± 3.7)
Specific heterosis	
Red River × Norris	8.6
Red River × MxK	2.1
Norris × M × K	2.2
	(± 5.2)
Maternal effects	
Red River	−6.1
Norris	21.6*
M × K	−15.4*
	(± 3.5)
Reciprocal effects	
Red River × Norris	5.3
Red River × M × K	3.9
Norris × M × K	−27.1*
	(± 3.7)
General combining ability	
Red River	3.8
Norris	−12.0*
M × K	8.2*
	(± 3.5)

*Significant at $P > 0.05$.

There was a significant difference in survival among catfish crosses following *E. ictaluri* exposure. Overall survival averaged 62.2 ± 4.9% and ranged from 35.8 ± 5.8% in the M × K female × Norris male cross to 90.0 ± 1.5% in the Norris female × M × K male cross

(Table 1). There was no significant difference in fish size among crosses, and no regression of mean weight on survival was necessary (Wolters and Johnson, 1994).

Line effects were significant in Norris and M × K strains (Table 2). Average or specific heterosis effects were not significant. Maternal effects were significant and negative in the M × K strain and positive in the Norris strain. Reciprocal effects were negative and significant in the Norris × M × K crosses. General combining ability was significant in both the Norris and M × K strains.

4. Discussion

The pattern of channel catfish mortality observed for the nine crosses in this study was consistent with previous *E. ictaluri* challenges (Newton et al., 1989; Thune and Johnson, 1992; Wolters and Johnson, 1994). Except for the Red River strain (Wolters and Johnson, 1994), no studies have reported relative survival following *E. ictaluri* for the other eight crosses used in this study. The average survival of the Red River strain in this study (56.5%), is lower than the survival (85.1%) reported in previous strain challenges (Wolters and Johnson, 1994). Numerical survival values obtained from different experimental disease studies should not be compared because of varying challenge conditions and fish origin. Relative ranking or survival of genotypes can only be compared within a specific challenge experiment.

Although crossbreeding has been reported to increase resistance to bacterial, viral, and parasitic infections in previous studies (Dunham and Smitherman, 1984; Dunham and Smitherman, 1987; Plumb et al., 1975), crossbreeding the three strains in this study did not result in significant heterosis for ESC resistance. Lack of significant heterosis implies no directional dominance for disease resistance, and the utilization of a crossbred strain (M × K) may have also caused decreased heterosis. Additional strains could yield different results and possibly detect superior crosses with ESC resistance.

Significant line effects, influenced by both additive and dominance genetic variation (Eisen et al., 1983), indicates strain differences and shows potential for improving ESC resistance through selection among strains. Although no heritability estimates for resistance to *E. ictaluri* have been made from sib analyses, Dunham et al., 1994 obtained no selection response and realized heritability for resistance to *E. ictaluri* infection in the Kansas strain. This strain has been propagated from broodfish originally collected in 1911 and the stock at Auburn was derived from six to eight pairings in 1976. The additive genetic variation in the Kansas strain may be low or already exploited and explain the lack of selection response for resistance to ESC. The M × K strain used in this study, however, had significant general combining ability indicating superior additive genetic merit. Other populations or strains could have more additive genetic variation and respond to selection for resistance to ESC.

Maternal effects, estimated as the difference between means of crosses involving females of a strain and means of crosses involving males of the same strain, were significant and positive in the Norris and negative in the M × K strains. Maternal effects in channel catfish convey increased or decreased disease resistance from differences in egg size, egg quality, and cytoplasmic inheritance (Gjerde, 1988). Reciprocal effects were also significant and

negative in Norris × M × K cross further indicating that the maternal genotype and mitochondrial DNA impacts ESC resistance.

Overall results document differences in ESC resistance among crosses of three catfish strains due to additive genetic merit and maternal effects. Poor performance was found for the Norris strain in crosses for ESC resistance, however, the positive significant maternal effects in that line show the potential for using Norris strain females in crosses with a male line having specific combining ability. The M × K strain had the best overall performance because of significant line effects and general combining ability. The M × K strain has shown superior growth and reproductive performance in previous studies (Dunham and Smitherman, 1987), and had better ESC resistance compared to the other two strains utilized in this study.

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